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Studies on Virus Diseases of Fishes. Spontaneous and Experimentally Induced Cellular Hypertrophy (Lymphocystis Disease) in Fishes of the New York Aquarium, with a Report of New Cases and an Annotated Bibliography (1874-1965)

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(Plates I-X)

LYMPHOCYSTIS disease, a non-lethal viral disease first (25) observed in European flounder in 1874, is characterized externally by the development of nodules on the fins and skin (Figs. 1-5, 7-9); the growths may also appear as tumor-like masses (Fig. 6) or as flat, confluent patches in various parts of the body (Figs. 10, 11). The lesions have a granular appearance due to numerous white, spherical or oval, tremendously enlarged connective tissue cells, lying singly or in groups in lymph spaces below the stratified epithelium (Figs. 12, 13, 14, 20, 23). These giant cells may also be found in the gills, pharynx, ovary, spleen and in the walls of the heart and gastro-intestinal tract (7, 35); the lymphocystis cells in these organs are probably displaced elements but their development *in situ* (Fig. 14) is possible. The lymphocystis cells are considered to be enlarged transformed fibroblasts (24, 63-67), but the possibility that they are hypertophied osteoblasts or histiocytes is not excluded.

The transformed connective tissue cell, which may enlarge from 10-15 microns to 500 microns or more (an increase in volume of about a million times) is typically surrounded by a hyalin capsule (Figs. 14, 17, 18). The cell contains an enlarged nucleus usually in various stages of karyorrhexis, one or more enlarged nucleoli and a densely granular, sometimes vacuolated, cytoplasm with basophilic, Feulgen-positive (19) inclusions, which may appear as a single, branching, perinuclear network or as several vacuolated plaques (Figs. 13-23). The inclusions arise from one or more Guarnieri-like bodies, usually seen in the smallest infected cells (1, 63, 64, 72, 73). Basophilic granules or rods that develop from the surface of the inclusions (74, 75, 76, 83-86) are readily visible as osmiophilic granules with the light microscope, and show characteristic viral morphology in electron-microscopic preparations (58, 60, 61). The osmiophilic particles may also be found in the nucleus and/or free in the cytoplasm of the enlarged cell (86). The inclusion particles, readily visible in ordinary histological preparations (Figs. 18, 24), suggest a cytomegalovirus.

Although lymphocystis disease is recognized in general by the enlargement of the connective tissue cell, gross and subtle species or family similarities or differences are apparent in certain cellular details (Figs. 13, 15, 20, 23, 25), which have not as yet been characterized. Some obvious differences are 1) maximum size that the infected cell can attain, 2) origin, structure and distribution of the basophilic inclusions, and 3) the electron-microscopic morphology and size of the virus particles.

Cellular distention appears to be a characteristic feature in certain viral infections in higher vertebrates (11). The enlargement in these instances is moderate when compared to the lymphocystis cell[1] which, in some cases (*e.g.*, in diseased cells of the European flounder), can increase a million-fold or more in volume. The factors responsible for this tremendous enlargement have not been determined but cannot be explained solely on viral multiplication, since the amount of basophilic inclusions varies con-

[1] "Glugea-cysts" is another form of cellular gigantism, but caused by intracellular protozoan parasites belonging to the Microsporidia (68, 69, 81).

siderably with the host cells of similar size in the same lesion. Some of the increase in size may be due to imbibition.

The histopathological changes associated with the development of the hypertrophied cells are usually mild (34-35). Early changes are manifested by varying degrees of inflammatory responses. In more advanced stages of the disease, the inflammatory reaction disappears and is replaced by a mild development of collagenous fibers. Later, the overlying epithelium, which may or may not be hyperplastic, is sloughed. The contents of the cells eventually empty, leaving only collapsed membranes and fibrous tissue; healing, however, is usually complete with no evidence of infection or scar tissue.

The origin and exact nature of the hyalin capsule, a distinctive feature of the lymphocystis cell, has not been firmly established. This material is PAS-positive (Fig. 26), indicative of mucoproteins, and the evidence suggests that it is secreted by the individual infected connective tissue cell (Figs. 14, 17), even though the cell itself appears to be PAS-negative (Fig. 26). When the cells are immediately adjacent to each other, the membranes become fused (Figs. 16, 20) or form, as in the case of the extreme development of lymphocystis cells in the striped bass, a diffused gelatinous-like matrix (Fig. 25).

The infectious nature of the disease, first recognized by Weissenberg (63) more than fifty years ago, was repeatedly demonstrated by him (64-67, 71-73, 79-80, 87) and others (33), and especially by the meticulous infection experiments by Rasin (43, 44)[2] and more recently by Wolf (93) and co-worker (94). The viral etiology proposed by Weissenberg (63) in 1914, and long accepted as the cause of lymphocystis disease purely on circumstantial evidence (11, 18, 33, 50, 51), was finally and firmly established in recent years by filtration and transmission experiments (79, 93, 94), and the virus particles identified and verified by the excellent electron-microscopic studies by Walker and his collaborators (58-61). Diseased tissue (virus) retains its infectivity even after storage at –20° C for two years, after desiccation of 105-days-old nodules over KOH for six days at 18-20° C, and after putrefaction in aquarium water for five days at 18-20° C. Further, a saline suspension of emulsified lymphocystis tissue has been found to be infective in dilution up to 1: 1 million (43, 44). The virus is glycerol- and ether-sensitive (93).

[2]Rasin also transmitted the disease from paradisefish to giant gourami, this being the first successful intergeneric transmission.

It is assumed that under natural conditions the disease is transmitted by the ingestion of lymphocystis cells or by contact with the contents that may be released into the environment when the cells burst (77). It has been suggested that the virus gains entrance into a new susceptible host by way of the gills (73, 80) but fishes that are scarred or possess open wounds are definitely more susceptible (37, 43, 44, 93). Protozoan, helminthic and crustacean (copepods and argulids) ectoparasites, including blood-sucking leeches, may play a direct role in the transmission of the disease, or the lesions caused by these parasites may be the foci for the penetration of the virus.

In so far as is known, Table I lists the host species in which lymphocystis disease has been reported (see annotated bibliography). It is quite evident from this table that the members of the Order Perciformes, or perch-like fishes, are especially susceptible. This, however, may not reflect a true picture, since the Perciformes is the largest order of fishes, comprising more than 160 families in several sub-orders. Forty-nine species of fishes, from 5 orders and 20 families, have been thus far reported in which the disease had developed spontaneously. Of interest in this list is the report by Templeman (53) that the disease also occurs in the North American plaice *(Hippoglossoides platessoides),* the first instance in a flatfish species from the western Atlantic. The following species in the New York Aquarium's collection are also new hosts for the disease: *Morone americana* (Fig. 10) from Tom's River, N.J., *Lepomis pallidus* from Connecticut, *Angelichthys ciliaris* from Florida, *Forcipiger longirostris* (Fig. 9) from Hawaii, *Aequidens pulcher* from Trinidad and *Scatophagus argus* (Fig. 3) from the Indo-Pacific. A second case of the disease in *Symphysodon discus* (Fig. 1) is also included.

Most of the species reported in Table 1 represent isolated cases, but relatively high incidence, sometimes reaching epizootic proportions, have been reported for flounders and other flat fishes in the English Channel, Irish, North, Baltic, Barents and Arctic Seas (5, 8, 36, 42) and for the perch or ruff (8, 64) from the streams of Middle and Northern Europe, including the brackish shores of the Baltic (Fig. 5). In North America, the disease is quite common in crappies from the eastern part of the United States (17, 91), and especially in the economically important walleye or pike perch from the Great Lakes and other lakes in which this species occurs (7, 16, 37, 46, 56, 72). Although the disease is not lethal, the affected walleyes are unsightly and are discarded by commercial fishermen. In

Table 1. Spontaneous Lymphocystis Disease: Host List
(M, Marine; B, Brackish; F, Freshwater; *, New Records)

Species	Common Name	Locality	Author
Class: Teleostomi			
Subclass: Actinopterygii			
I. Order: Clupeiformes			
(1) Family: Osmeridae			
1. *Osmerus eperlanus* (B)	European Smelt	North and Baltic Seas	2, 14
II. Order: Cyprinodontiformes			
(2) Family: Cyprinodontidae			
2. *Fundulus heteroclitus* (B)	Common Killifish	North Atlantic	71
III. Order: Perciformes			
(3) Family: Serranidae			
3. *Roccus lineatus* (B)	Striped Bass	North Atlantic	32 (N.Y.A.)[1]
4. *Serranus atricauda* (M)		Mediterranean	41
5. *Morone americana** (B)	White Perch	Eastern U.S.	(N.Y.A.)[2]
(4) Family: Centrarchidae (F)			
6. *Lepomis gibbosus*	Pumpkinseed Sunfish	Eastern U.S.	32, 73 (N.Y.A.)
7. *L. macrochirus*	Bluegill Sunfish	..	59-61, 73, 92-94 (N.Y.A.)[3]
8. *L. pallidus**		..	(N.Y.A.)[4]
9. *L. humilis*	Red-spotted Sunfish	..	31, (N.Y.A.)
10. *L. megalotis*	Long-ear Sunfish	..	50, 73
11. *L. cyanellus* × *L. macrochirus*	Blue-spotted × Bluegill	..	73
12. *Pomoxis annularis*	White Crappie	..	17, 91 (N.Y.A.)[5]
13. *P. nigromaculatus*	Black Crappie	..	17, 73, 91, (N.Y.A.)[5]
14. *Micropterus pseudaplites*	False Large-mouth Bass	..	73
15. *M. (Huro) salmoides*	Large-mouth Bass	..	73 (N.Y.A.)[6]
(5) Family: Percidae			
16. *Acerina cernua* (B)	Ruff or European Perch	Baltic and North Seas	2, 8, 63, 64 74, 85 (N.Y.A.)[7]
17. *Perca flavescens* (F)	Yellow Perch	Eastern N.A.	54
18. *Stizostedion vitreum* (F)	Walleye or Pike-perch	..	6, 7, 12, 16, 18, 26, 31, 33, 37, 38, 46, 54-60, 62, 72, 74 79, 80, 85, 92 (N.Y.A.)[8]
19. *S. canadensis griseus* (F)	Sauger	..	7
20. *S. glaucum* (F)	Blue Pike	..	7
(6) Family: Mullidae			
21. *Mullus surmuletus* (M)	Red Mullet	English Chan.	1, 2
(7) Family: Sparidae			
22. *Sargus annularis* (M)	Sargo	Adriatic Sea	8, 14, 23, 24
(8) Family: Chaetodontidae (M)			
23. *Chaetodon striatus*	Banded Butterfly Fish	Florida and Bahamas	31 (N.Y.A.)
24. *Pomacanthus arcuatus*	Black Angelfish	..	31, 32, 78 (N.Y.A.)
25. *Pomacanthus paru*	French Angelfish	..	78
26. *Angelichthys isabelita*	Blue Angelfish	..	30, 52 (N.Y.A.)
27. *Angelichthys ciliaris**	Queen Angelfish	..	(N.Y.A.)[9]
28. *Forcipiger longirostris**	Forceps Fish	Hawaii	(N.Y.A.)
(9) Family: Pomacentridae (M)			
29. *Amphiprion percula*	Common Clownfish	S. Pacific	9, 30 (N.Y.A.)
30. *Premnas biaculeatus*	Spiny Clownfish	..	9, 49
(10) Family: Scatophagidae			
31. *Scatophagus argus** (B)	Scat	Indo-China	(N.Y.A.)
(11) Family: Cichlidae (F)			
32. *Cichlosoma synspilum*		Guatemala	78, 88
33. *Aequidens portalegrensis*	Port or Black Acara	S. America	33 (N.Y.A.)[10]
34. *Aequidens pulcher**	Blue Acara	Trinidad	(N.Y.A.)[10]

TABLE 1. SPONTANEOUS LYMPHOCYSTIS DISEASE: HOST LIST—(*continued*)
(M, Marine; B, Brackish; F, Freshwater; *, New Records)

Species	Common Name	Locality	Author
35. *Symphysodon discus*	Discus; Pompador Fish	S. America	40 (N.Y.A.)
(12) Family: Labridae			
36. *Lachnolaimus maximus* (M)	Common Hogfish	Florida	2, 89 (N.Y.A.)
(13) Family: Blennidae			
37. *Hypsoblennius gentilis* (M)		S. Calif.	78
38. *H. jenkinsi* (?) (M)		..	82
(14) Family: Anabantidae			
39. *Macropodus opercularis* (F) (= *M. viridiauratus*)	Paradisefish	S. China	8, 14, 15, 43, 44, 48, 96. Not 90.
(15) Family: Eleotridae			
40. *Dormitator maculatus* (F)	Sleeper	Mexico	31 (N.Y.A.)[11]
(16) Family: Hexagrammoidae			
41. *Ophiodon elongatus* (M)	Blue Cod	British Col.	54
IV. Order: Pleuronectiformes			
(17) Family: Pleuronectidae			
42. *Pleuronectes flesus* (M)	European Flounder	Irish, North, Baltic, Barents Arctic Seas	2-5, 8, 10, 13, 14, 19-22, 25, 27-29, 36, 39, 42, 45, 47, 63-67, 74, 83, 84, 86, 88, 95
43. *Pl. platessa* (M)	Plaice	English Chan. & North Sea	2, 8, 14, 20, 21, 25, 27, 36, 47-49, 63, 95
44. *Pl.* (= *Limanda*) *limanda* (M)	Dab	..	2, 8
45. *Hippoglossoides platessoides* (M)	American Plaice	N. Atlantic, Newfoundland	53
(18) Family: Soleidae			
46. *Solea vulgaris* (M)	Common Sole	North Sea	2, 8, 14, 20
V. Order: Tetraodontiformes			
(19) Family: Monacanthidae			
47. *Ceratacanthus* (= *Aleutera*) *schoepfii* (M)	Orange Filefish	Atlantic Coast	30, 35, 70 (N.Y.A.)
(20) Family: Ostraciidae			
48. *Lactophrys tricornis* (M)	West Indian Cowfish	Florida and Bahamas	31, 33 (N.Y.A.)[12]
49. *L. cornutus* (M)	East Indian Cowfish	Indian Ocean	31 (N.Y.A.)

[1]From Drs. Roland Smith (N.J. State Conservation Dep't), A. Perlmutter (N.Y. State Conservation Dep't, Marine Division, and N.Y.U.), D. Merriman (Bingham Oceanogr. Lab., Yale Univ.).
[2]From Dr. B. Levine (Tom's River, N.J.).
[3]From Dr. Allison (Univ. Alabama).
[4]From Dr. C. P. Helmbolt (Univ. Conn.).
[5]From Dr. D. Hansen (Ill. Nat. Hist. Survey, Univ. Ill.).
[6]From Dr. Roland Smith.
[7]From Dr. Richard Weissenberg (Phila., Penn.).
[8]From Drs. R. V. Bangham (Coll. of Wooster) and Louis A. Krumholz (Univ. Louisville).
[9]From Dr. Wm. Braker (Shedd Aquarium, Chicago).
[10]From Mr. E. Weiss (tropical fish dealer, Brooklyn, N.Y.).
[11]From Dr. Myron Gordon (deceased) (Genetics Lab., N.Y. Aquarium).
[12]From C. M. Breder, Jr. (former director, N.Y. Aquarium).

addition, these fish are often simultaneously affected with a neoplastic disease (fibro-sarcoma) (55-57) (Fig. 6). Attention is also called to the high incidence (20-30%) of multiple tumors in pike perch in certain lakes of the USSR during the summer months, for which a virus is suspected as the cause (38).

Lymphocystis disease is quite common in the orange filefish (35, 70) during their summer residency along the north Atlantic Coast, and in striped bass (33) off the coast of New Jersey, New York and Connecticut, particularly in the spring and early summer. The disease in the white crappie and pike perch also shows a seasonal distribution, with the highest incidences occurring during the spring spawning runs (17, 46, 91).

Table 2 lists the host species in the New York

TABLE 2. EXPERIMENTALLY-INDUCED LYMPHOCYSTIS DISEASE IN FISHES IN THE NEW YORK AQUARIUM COLLECTION

Species	Common Name	No. Fish In Which Disease Was Transmitted	Remarks
Order: Perciformes			
(1): Family: Centrarchidae			
1. *Lepomis macrochirus*	Bluegill Sunfish	6	Spontaneous disease found in 1 fish in Bronx Zoo pond; 1 auto- and 5 homotransplant; positive takes in 10 days at 22° C; June, 1956.
(2) Family: Chaetodontidae			
2. *Forcipiger longirostris*	Forceps Fish	1	Disease found in 3 fish; 1 positive autotransplant in 8 days at 25° C; Jan., 1965.
(3) Family: Cichlidae			
3. *Aequidens portalegrensis*	Port or Black Acara	3	Disease found in 4 fish; 1 fish used as donor. Serially transplanted for 3 passages; 4th passage negative; disease developed in 10 days at 25°C. Infected fish designated port no. 1, 2 & 3; Jan., 1952.
4. *Aequidens pulcher*	Blue Acara	3	Port No. 1 used as donor; 2 positive and 1 negative; 12 days at 25° C.
5. *Hemichromis bimaculatus*	Fire-mouth[1]	1	Port No. 1 donor; 1 positive and 3 negative; homotransplant negative; 10 days at 25° C.[2]
6. *Tilapia macrocephala*	Black-chinned Mouth-breeder[1]	1	Port No. 1 donor; 3 positive; homotransplant negative; 12 days at 25° C.[2]
7. *Tilapia ovale*	Oval Tilapia[1]	1	Port No. 1 donor; 1 positive; homotransplant negative; 11 days at 25° C.[2]
8. *Tilapia sparmanii*	Sparman's Tilapia[1]	2	Port No. 1 donor; 2 positive, 1 negative; 13 days at 25° C.

[1]Disease not previously described for these species.
[2]Fish donated by Dr. L. Aronson, American Mus. Nat. History.

Aquarium's collection in which lymphocystis disease was experimentally induced. Except for *Aequidens portalegrensis* and *A. pulcher*, the cichlids listed have not been reported as actual or potential hosts for this disease. The transmission experiments were made simply by implanting fragments of lymphocystis tissue into the pockets from which the scales were removed, or by intradermal injection of emulsified material on one side of the same host (Fig. 9) or in another fish of the same (Fig. 8) or different species. Lesions typical of lymphocystis disease usually appear in 10 to 12 days at approximately 22-25° C, temperatures at which these fish are kept in the New York Aquarium. The incubation period and the rate of development of the disease is temperature-dependent (44, 93, 94). In certain species, the disease may persist for five to six months (44), or longer, and we have seen lymphocystis cells appear and disappear within a few days. Lymphocystis disease can be serially transplanted for a limited number of passages (44, 94), and apparently there is a certain degree of host resistance (35, 79, 93, 94), either natural or acquired, as indicated by some of the experiments shown in Table II.

SUMMARY

Forty-nine species of fishes, 20 families from 5 orders, with spontaneous lymphocystis disease (viral induced cellular hypertrophy or cellular gigantism) are reported. Twenty-six of these diseased species, in 11 families and 2 orders, were

found in the New York Aquarium's collection. New host records are: *Morone americana* (white perch) from Tom's River, New Jersey, *Lepomis pallidus* from Connecticut, *Angelichthys ciliaris* (queen angelfish) from Florida, *Forcipiger longirostris* (forceps fish) from Hawaii, *Aequidens pulcher* (blue acara) from Trinidad and *Scatophagus argus* (scat) from the Indo-Pacific. A second case in *Symphysodon discus* (discus or pompador fish) from the Amazon Basin is also reported.

Experimental transmission of the disease is recorded for the following cichlids in which the disease has not been previously reported: *Hemichromis bimaculatus* (fire-mouth), *Tilapia macrocephala* (black-chinned mouth-breeder), *Tilapia ovale* (oval Tilapia), and *Tilapia sparmanii* (Sparman's Tilapia).

Lymphocystis disease is briefly described and an annotated bibliography (1874-1965) is included.

Annotated Bibliography

1. Alexandrowicz, J. S.
   1951. Lymphocystis Tumours in the Red Mullet *(Mullus surmuletus* L.*)*. J. Mar. Biol. Assoc., U.K., 30: 315-332.
   Excellent histological description of the lymphocystis cells; speculations on the events leading to release of infective virus.

2. Amlacher, Erwin
   1961. Taschenbuch der Fischkrankheiten. Gustav Fischer, Jena., 286 pp.
   Brief description of the disease. Species listed are: flounder, plaice, sole, dab, ruff, red mullet, smelt, angelfish and hogfish.

3. Awerinzew, S.
   1907. Zur Kenntnis von *Lymphocystis johnstonei* Woodcock. Zool. Anz., 31: 881-884.
   Henneguya johnstonei is a new name for *Lymphocystis johnstonei.*

4. 1909. Studien über parasitische Protozoen. II. *Lymphocystis johnstonei* Woodc. und ihr Kernapparat. Arch. f. Protist., 14: 335-362.
   Disease in *Pleuronectes flesus* from Barents Sea. The enlarged cells were described as protozoans.

5. 1911. Studien über parasitische Protozoen. V. Einige neue Befunde aus der Entwicklungsgeschichte von *Lymphocystis johnstonei* Woodc. Arch. f. Protist., 22: 179-196.
   11% of flounders *(Pleuronectes flesus)* from the Murmansk Coast (Arctic Sea) affected annually; intracellular structures reported as stages in the development of a myxosporidian spore.

6. Bangham, Ralph V.
   1946. Parasites of Northern Wisconsin Fish. Trans. Wisc. Acad. Sci., Arts and Lett., 36 (1944): 291-325.
   Lymphocystis in walleye pike from Wisconsin Lakes.

7. Bangham, Ralph V., & G. W. Hunter, III
   1939. Studies on Fish Parasites of Lake Erie. Distribution Studies. Zoologica, 24: 383-448.
   Lymphocystis disease in the following: 7 walleye pike *(Stizostedion vitreum)*, 1 blue pike *(Stizostedion glaucum)* and 1 sauger *(Stizostedion canadensis griseus)*; lesions mainly on fins. Lymphocystis cells in walleyes also found in wall of digestive tract and heart.

8. Bergman, Arvid M.
   1922. Fiskarnas Sjukdomar. Albert Bonniers Forlag, 73 pp.
   Lymphocystis disease in flounders from Swedish and Danish waters; excellent photographs of the lesions and a drawing of "mature" lymphocystis cell from the ruff. Disease is found annually in 5% of the ruff and in 6-11% of the flounder from the Baltic Sea. Other species referred to are: plaice *(Pleuronectes platessa)*, dab *(Pleuronectes limanda)*, sole *(Solea vulgaris)*, paradisefish *(Macropodus viridiauratus)* and the sargo *(Sargus annularis)*.

9. Benisch, J.
   1937. Über das Auftreten der Lymphocystis-Krankheit bei einigen Korallenfischarten. Wochenschr. f. Aq. und Terrarienkunde, 34: 380-382.
   First report of lymphocystis in the coral fishes *(Premnas biaculeatus* and *Amphiprion percula)*; photographs included.

10. Claussen, K.
    1917. Über Knotchenformigen Hautauschlag bei Flundern. Zeitschr. f. Fleisch. -u-Milchhyg, 27: 241.
    Lymphocystis in *Pleuronectes flesus* from the North Sea; agrees with Weissenberg as to the nature of the cells.

11. Cowdry, E. V.
    1955. Cancer Cells. W. B. Saunders Co., Philadelphia, 677 pp.
    Discusses lymphocystis cells in relation to cellular gigantism in cancer generally and the role of viruses in certain neoplastic diseases.

12. Davis, H. S.
    1953. Culture and Diseases of Game Fishes. Univ. of California Press, 332 pp.
    Brief description of the disease and notes its absence in salmonids.

13. Doflein, F.
    1928. Lehrbuch der Protozoenkunde. II. Teil. Verlag von G. Fischer, Jena, pp. 439-1262.
    On page 1136, discusses *Lymphocystis john-*

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15. Dyk, Václav
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16. Fischthal, J. H.
 1947. Parasites of Northwest Wisconsin Fishes. Trans. Wisc., Acad. Sci., Arts & Lett., 37: 157-220.
 Disease in walleye pikes in Upper Turtle and Teal Lakes, Wisconsin.

17. Hansen, Donald
 1951. Biology of the White Crappie in Illinois. Bull. Ill. Nat. Hist. Sur., 25: 211-265.
 Disease on the fins of black and white crappies, *Pomoxis nigromaculatus* and *Pomoxis annularis*, from bottom land lakes in the Illinois River Valley. Incidence of the disease in the white crappie: 1.4% (Senachwine Lake, April, 1942), 9.5% (Lake de Pue, April 25-27, 1942), 19.5% (Lake Chautauqua, Sept. 17-18, 1943).

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 Similar report.

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24. 1918. Untersuchungen über Lymphocystis Woodc. Arch. f. Protist., 38: 155-249.
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25. Lowe, John
 1874. Fauna and Flora of Norfolk. Part IV. Trans. Norfolk and Norwich Nat. Soc., Fishes. pp. 21-56.
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26. Mavor, J. W., & S. M. Feinberg
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 First report of lymphocystis disease in the New World, but the cells were interpreted as protozoan parasites.

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 1885. Diseases of Fishes. I. Multiple Tumours in Plaice and Common Flounder. 3rd Ann. Rept. Scot. Fish. Bd. for 1884., p. 66-67.
 The lesions were later recognized as lymphocystis tumors.

28. 1886. Further Remarks on the Multiple Tumours of Common Flounder. 4th Ann. Rept., Scot. Fish. Bd. for 1885, p. 214-215.
 Additional report on multiple tumors, including the lesions later reported as *Lymphocystis johnstonei* by Woodcock.

29. Minchin, E. A.
 1912. An Introduction to the Study of Protozoa, with Special Reference to the Parasitic Forms. Ed. Arnold Publ., London, 517 pp.
 First to doubt the protozoan nature of *Lymphocystis johnstonei* Woodcock from flounders.

30. Nigrelli, Ross F.
 1940. Mortality Statistics for Specimens in the New York Aquarium, 1939. Zoologica, 25: 525-552.

Lymphocystis in 1 clownfish *(Amphiprion percula)*, 2 blue angelfish *(Angelichthys isabelita)* and 1 orange filefish *(Ceratacanthus schoepfii)*.

31. 1943. Causes of Disease and Death of Fishes in Captivity. Zoologica, 28: 203-216.
Lymphocystis found in 1940 in the following species: 1 banded butterfly fish *(Chaetodon striatus)*, 2 black angelfish *(Pomacanthus arcuatus)*, 1 East Indian cowfish *(Ostracion cornutus)*, 1 West Indian cowfish *(Lactophrys tricornis)*, 1 red-spotted sunfish *(Lepomis humilis)*, 3 striped sleeper *(Dormitator maculatus)*, 6 pike-perch *(Stizostedion vitreum)*.

32. 1950. Lymphocystis Disease and Ergasilid Parasites in Fishes. J. Para., 36: 36.
Disease in black angelfish, striped bass *(Roccus lineatus)*, and pumpkinseed sunfish *(Lepomis gibbosus)* in the New York Aquarium. The role of copepod parasites briefly discussed.

33. 1952. Virus and Tumors in Fishes. Ann. N. Y. Acad. Sci., 54: 1076-1092.
Photographs of lymphocystis disease in West Indian cowfish, pike-perch and in cichlid *(Aequidens portalegrensis)*. Successful transmission from diseased cichlid to healthy fish of the same species by direct implantation.

34. 1954. Tumors and Other Atypical Cell Growths in Temperate Freshwater Fishes of North America. Trans. Amer. Micro. Soc., 83: 262-295.
North American host list and a brief description of the disease.

35. Nigrelli, R. F., & G. M. Smith
1939. Studies on Lymphocystis Disease in the Orange Filefish *Ceratacanthus schoepfii* (Walbaum), from Sandy Hook Bay, N.J. Zoologica, 24: 255-264.
A review of the literature, together with a detailed macroscopic and microscopic description of the disease in the filefish, a summer resident in the New York Bight. Lymphocystis cells found in fins, ovary, spleen and wall of the gastro-intestinal tract; stages in healing process described. Attempts to transmit the disease to killifish were negative.

36. Nordenberg, Carl-Bertel
1962. Das Vonkommen der Lymphocystiskrankheit bei Scholle und Flunder im Oresund. Kungl. Fysiogr. Sallskijets i Lund Forhandl., 32: 17-26.
Incidence of disease in flounders: April-May 7%; July-Sept. 10-12%; Oct.-Dec. 3-4%; Jan.-March 7-8%.

37. Olsen, Donald E.
1958. Statistics of a Walleye Sport Fishery in a Minnesota Lake. Trans. Amer. Fish. Soc., 87: 52-72.
Fish that were marked by removing scales or by clipping fins were more susceptible to the disease.

38. Petrushevskii, G. K.
1957. Parasites and Diseases of Fish. Bull. All-Union Sci. Res. Inst. Fresh-water Fisheries (USSR), 42: 1-338. (1961 Translation publ. for NSF and U.S. Dept. Int. by the Israel Program for Scientific Translation).
Multiple tumors in 20-30% of the pike-perch in White Lake (USSR) during the summer suspected to be viral in origin; the tumors may be lymphocystis.

39. Plehn, M.
1924. Praktikum der Fischkrankheiten. E. Schweizerbart'sche, Stuttgart, 179 pp.
Brief description of the disease in European fishes.

40. Porter, Annie
1952. Report of the Honorary Parasitologist for the year 1951. Proc. Zoological Society of London, 122: 535-536.
Lymphocystis in the disc cichlid *(Symphysodon discus)*.

41. 1953. Report of the Honorary Parasitologist for the year 1952. Proc. Zoological Society of London, 123: 253-257.
The disease in five striped sea perch *(Serranus atricauda)*.

42. Raabe, H.
1935. Un *Microsporidium* dans des *Lymphocystis* chez les plies. Bull. de l'Institut Oceanographique (Monaco), No. 665: 1-10.
Disease in flounders *(Pleuronectes flesus)* from the Baltic near the Marine Station at Hel (Poland) in the Bay of Dantzig. High incidence of lymphocystis found annually in the spring. The development of the gigantic cell, characteristic of lymphocystis, believed to be caused by an intracellular microsporidium.

43. Rasin, K.
1927. Prispevek k pathogenesi *Lymphocystis johnstonei* Woodcock. I. Biol. Spisy Vysoké Skoly Zvérolekarské Brno. (Publ. Biol. Ecole Vet.), 6: 11-38. (Biol. Abstract no. 24008, 1931).
First detailed experimental studies on transmission of lymphocystis disease in paradisefish, *Macropodus*. Infection induced by implantation, by injection of saline suspension of emulsified diseased tissue, by smearing injured skin with emulsion and by exposing injured (scaled) fish to emulsified material introduced to tank water; lymphocystis tissue 47 to 105 days old infective, even 105-day-old material dried over KOH at 18-20° C, but with diminished virulency after 67 days of drying. Lymphocystis disease in *Macropodus* disappears in 5-7 months.

44. 1928. II. *idem., ibid*, 7: 1-14. (Biol. Abstract No. 20631, 1931).
Virus (emulsion) causing lymphocystis disease in *Macropodus* carried through 17 passages. Lymphocystis material from fish dead for 24 hrs. produced disease in healthy fish in 9 days; material obtained from fish allowed to putrefy for 5 days in tank water at 18-20° C retained

its infectivity; severity of disease related to dilution and to length of exposure time; 1:1 million dilution of suspension of emulsified material infective. Rate of growth of lymphocystis cells in experimental fish related to temperature; cells double in size in 12 days at 30° C compared to growth of cells in fish in 24 days at 18° C; fish kept at 16° C more resistant to experimental infection; susceptibility of fish to lymphocystis is increased by direct application of virus (emulsion) to damaged skin. Inability to demonstrate filterability of virus believed to be due to absorption of virus on tissue fragments which were removed by the filter paper. Giant gourami *(Trichogaster fasciatus= Colisa fasciata)* experimentally infected with lymphocystis from paradisefish *(Macropodus);* this represents *first* inter-generic transmission.

45. Reichenbach-Klinke, H.-H.
1957. Krankheiten der Aquarienfische. Alfred Kernen Verlag, Stuttgart, 215 pp.
Short discussion of lymphocystis disease.

46. Ryder, R. A.
1961. Lymphocystis as a Mortality Factor in a Walleye Population. The Progressive Fish-Culturist, 23: 183-186.
Walleye population from Nipigon River, Ontario. In 1956, 1,000 fish were tagged, 248 (24.8%) of which were diseased; 55 (22.2%) of the diseased fish were recaptured. In 1957, 504 fish were tagged, 147 (29.2%) of which were infected, and 52 (35.4%) of these were recaptured. Incidence of the disease increased during the spawning period from 17.5% at the start of the tagging to 30.5% at its termination 10 days later; lymphocystis at its highest level during and immediately after spawning; tagged infected fish showed no trace of the disease in summer, fall or winter. No appreciable effect on mortality rates; diseased fish more susceptible to capture by gill nets.

47. Sandeman, G.
1893. On the Multiple Tumours in Plaice and Flounders. 11th Ann. Rept. Scot. Fish. Bd. for 1892, 391-392.
Most of the multiple tumors in these fish were lymphocystis.

48. Schäperclaus, W.
1935. Fischkrankheiten. G. Wenzel u. Sohn, Braunschweig, 72 pp.
Excellent photographs of the lesions on fins of a plaice and on a female paradisefish.

49. 1954. Fischkrankheiten. Akademie-Verlag, Berlin, 708 pp.
Photographs of lymphocystis cells of the plaice, and the lesions *in situ* in the clownfish, *Premnas biaculeatus,* reported by Benisch (1937).

50. Schlumberger, Hans G.
1958. Krankheiten der Fische, Amphibien und Reptilien. *In:* Vol. II. Pathologie der Laboratoriumstiere. Springer-Verlag, Berlin.
Excellent macroscopic and microscopic photographs of lymphocystis in the long-ear sunfish, *Lepomis megalotis.*

51. Smith, Kenneth
1940. The Virus. Macmillian Co., N. Y., 176 pp.
Lists lymphocystis among the known viral diseases of plants and animals.

52. Smith, G. M., & R. F. Nigrelli
1937. Lymphocystis Disease in *Angelichthys.* Zoologica, 22: 293-295.
First report of lymphocystis disease in a marine fish *(Angelichthys isabelita)* of the North American Atlantic coast.

53. Templeman, Wilfred
1965. Lymphocystis Disease in American Plaice of the Eastern Grand Bank. Jour. Fisheries Res. Board of Canada, in press.
First description of lymphocystis disease in North American flat fishes *(Hippoglossoides platessoides);* 1% of the fishes found to be infected.

54. Walker, Roland
1947. Lymphocystis Disease and Neoplasia in Fish. Anat. Rec., 99: 559-560. (abstract).
First report of lymphocystis in a Pacific Coast fish, *Ophiodon elongatus,* from the Straits of Georgia, B.C.,; also in 5 yellow perch, *Perca flavescens,* from Lake Erie and in walleye pike from Lake Oneida, in which lymphocystis is associated with sarcomatous tumors.

55. 1957. Warty Walleyes. The N. Y. State Conservationist, 12: 28-29.
Lymphocystis and sarcoma in walleyes from Lake Oneida, New York.

56. 1958. Lymphocystis Warts and Skin Tumors of Walleye Pike. Rensselaer Review of Graduate Studies, No. 14: 1-5.
The incidence of the disease in walleye pike from Lake Oneida ranges from less than 1% to 5%.

57. 1961. Fine Structure of a Virus Tumor of Fish. American Zoologist, 1: (Abstract No. 71).
The virus of the sarcoma in walleye pike found to be different from the lymphocystis virus.

58. 1962. Fine Structure of Lymphocystis Virus of Fish. Virology, 18: 503-505.
First detailed electronmicroscopic report of the lymphocystis virus. The viral particles of the lymphocystis cells from the pike perch measure 200 millimicrons and show polyhedral capsids surrounded by nucleoids.

59. 1965. Viral DNA and Cytoplasmic RNA in Lymphocystis Cells of Fish. *In:* Viral Diseases of Poikilothermic Vertebrates. Annals N. Y. Acad. Sci., 126: 375-385.
Distribution of viral DNA and cytoplasmic RNA in lymphocystis cells from walleye pike

and sunfish *(Lepomis)* as revealed by U.-V. Fluorescence Microscopy after staining with acridine orange.

60. Walker, Roland, & R. Weissenberg
1965. Conformity of Light- and Electron- Microscopic Studies on Virus Particle Distribution in Lymphocystis Cells of Fishes. *Ibid.*, 126: 386-395.
The viral particles in lymphocystis cells from diseased walleye pike, bluegill sunfish, European flounder and the cichlid fish *(Cichlasoma)* are compared.

61. Walker, Roland, & Ken Wolf
1962. Virus Array in Lymphocystis Cells of Sunfish. American Zoologist, 2: 566 (Abstract).
Electron microscopic studies of lymphocystis cells in bluegill sunfish following subcutaneous inoculation with cell-free filtrate of homogenized diseased tissue from *Micropterus*. The virus particles are similar to those seen in walleye pike.

62. Watson, Stanley W.
1954. Virus Diseases of Fish. Trans. Amer. Fish. Soc., 83: 331-341.
A brief review of the fish species susceptible to lymphocystis disease. Lesions persist from 1 to 3 years; diseased walleyes from Saginaw Bay weighed less (5.5 to 6.5%) than "healthy" fish of the same length.

63. Weissenberg, R.
1914. Über infetiöse Zellhypertrophie bei Fischen (Lymphocystiserkrankung). Sitz.-Ber. Klg. preuss. Akad. Wiss., 30: 792-804.
First to recognize the infectious nature of disease in European flounder, plaice and ruff; that the disease is caused by a virus; that the enlarged elements are hypertrophied host connective tissue cells; that the inclusions are viral reaction products.

64. 1920. Lymphocystisstudien. (Infektiöse Hypertrophie von Stutzgewebszellen bei Fischen). I. Die reifen Geschwulste bei Kaulbarsch und Flunder. Lymphocystisgenese beim Kaulbarsch. Arch. mikr. Anat., 94: 55-134.
Detail description of the development of the lymphocystis tumor in the ruff, *Acerina cernua*, and flounder, *Pleuronectes flesus*, from the Baltic.

65. 1921a. Lymphocystisstudien. II. Abgrezung Netzkörpers der Lymphocystiszellen gegen das Golginetz (Joseph's centrophormium). Sitzunsber. Gesellsch. Naturforsch. Fruende Berlin, 1920 (vorgetragen in der Sitzung, vol. 15, Juni).
Discussion on the cytoplasmic network in the lymphocystis cell in relation to Joseph's interpretation that the network is a centrophormium or golgi-net.

66. 1921b. Neue Lymphocystisbeobachtungen. Sitzungsber. Berlin Mikrobiol. Gesselsch. (Kurze mitteilung in der Sitzung vom 7 Juni, 1920). Berlin Klin. Wochenschr., nr. 2: 35.
Additional studies on lymphocystis disease in European fishes and reiteration of the viral etiology.

67. 1921c. Lymphocystiskrankheit der Fische. *In:* S. v. Prowazek und W. Noller, Handbuch der Pathogenen Protozoen. 3: 1344-1380.
Review of lymphocystis disease in European fishes.

68. 1929. Neue Gesichtspunkte in der vergleichenden Tumorforschung. Zeitschr. f. arztliche Fortbildung, No. 17: 555-559.
Comparison of the cell hypertrophy due to virus as in lymphocystis disease of the flounder and ruff and that caused by intracellular microsporidians in ganglion cells of the angler fish *(Lophius piscatorius)*, and certain connective tissue cells of European sticklebacks.

69. 1937. Intracellular Parasitism in Fish Producing a Gigantic Growth of the Infected Cells. Anat. Rec., 70: 68. (Abstract).
Further comparison of lymphocystis virus and microsporidian parasites producing cellular gigantism of the host cells.

70. 1938. Studies on Virus Diseases of Fish. I. Lymphocystis Disease of the Orange filefish *(Aleutera schoepfii)*. Amer. J. Hyg., 28: 455-462.
Description of disease in a filefish in the Philadelphia Aquarium. Line drawings showing the development of the characteristic cytoplasmic inclusions.

71. 1939a. Studies on Virus Diseases of Fish. II. Lymphocystis Disease of *Fundulus heteroclitus*. Biol. Bull., 76: 251-255.
Description of the disease in the killifish. Attempt at infecting *Fundulus heteroclitus* and *F. diaphanus* with walleye pike lymphocystis material were negative; concluded that there is a certain degree of host specificity.

72. 1939b. Studies on Virus Diseases of Fish. III. Morphological and Experimental Observations on the Lymphocystis Disease of the Pike-Perch, *Stizostedion vitreum*. Zoologica, 24: 245-254.
Sixty diseased pike-perch collected from Lake Huron and Lake Erie. Detailed description of the disease in this species; transmission of the disease to young pike-perch from Spirit Lake, Iowa; 5% of pike-perch from Saginaw Bay, Lake Huron, were infected in Spring of 1937.

73. 1945. Studies on Virus Diseases of Fish. IV. Lymphocystis Disease in Centrarchidae. Zoologica, 30: 169-184.
Lymphocystis reported in following species: 18 *Lepomis gibbosus*, 21 *L. macrochirus*, 1 hybrid

L. cyanellus × *L. macrochirus*, 1 *L. megalotis*, 2 *Pomoxis nigromaculatus*, 3 *Huro salmoides*, 1 *Micropterus pseudaplites*. Also reports transmission experiments in *L. gibbosus* and *L. macrochirus;* disease appeared in 25 days after spraying lymphocystis emulsion over the gills; *Lepomis* not susceptible to lymphocystis from pike-perch. Development of Guarnieri-like body to perinuclear network described and figured.

74. 1946. Observations on the Developmental Cycle of the Lymphocystis Virus in Fish *(Pleuronectes flesus, Stizostedion vitreum, Acerina cernua)*. Anat. Rec., 94: 89. (Abstract).
Concludes that lymphocystis is caused by a macrovirus associated with plaques, somewhat like the elementary bodies in viral diseases of higher vertebrates.

75. 1949. Studies on Lymphocystis Tumor Cells of Fish. I. The Osmiophilic Granules of the Cytoplasmic Inclusions and Their Interpretation as Elementary Bodies of the Lymphocystis Virus. Cancer Research, (9): 537-542.
The osmiophilic granules on the outer layers of the lymphocystis inclusion bodies correspond to the elementary bodies of other macrovirus and are considered to be infective stages of the lymphocystis virus.

76. 1951a. Studies on Lymphocystis Tumor Cells of Fish. II. Granular Structures of the Inclusion Substance as Stages of the Developmental Cycle of the Lymphocystis Virus. Cancer Research, 11: 608-613.
Evidence suggests that non-osmiophilic granules of the inclusion substance multiply by fission and eventually form osmiophilic granules; the former are considered the vegetative stages which serve as growth for the virus within the host cells and the osmiophilic granules are the infective virus particles. The osmiophilic granules, which accumulate in large numbers as the cells increase in size, are liberated by disintegration of the tumor cells after they are extruded into the water, or after death of the fish.

77. 1951b. Some Results of Morphological Studies on the Developmental Cycle of the Lymphocystis Virus of Fish with Reference to the Experimental Work of K. Rasin. Anat. Rec., 111: (Abstract no. 187).
The bursting of lymphocystis cells *in situ* produces infection of neighboring fibroblasts or phagocytes.

78. 1951c. Four Additions to the List of Host Fish in Which Lymphocystis Tumors have been Observed as the Result of Spontaneous Viral Infection. Anat. Rec., 111: (Abstract no. 289).
Spontaneous lymphocystis found in 1 black angelfish *Pomacanthus arcuatus*, 1 French angelfish *Pomacanthus paru*, 1 *Hypsoblennius gentilis*, and 2 *Cichlasoma synspilum*.

79. 1951d. Experimental Lymphocystis Infection of the Killifish *Fundulus heteroclitus* with Emulsion of Lymphocystis Tumors of the Perch *Stizostedion vitreum*. Anat. Rec., 111: (Abstract no. 290).
Contrary to his 1939 (a) studies, positive infection of killifish occurred when exposed to tumor emulsion of *Stizostedion;* in 1944, 4 *Fundulus* of six fish treated became infected; in 1945, one out of 41 fish became infected; however, in both experiments, there was an early cessation of growth of the tumor cells followed by degeneration, indicating that the killifish is not a suitable host for pike-perch lymphocystis virus.

80. 1951e. Positive Result of a Filtration Experiment Supporting the View that the Agent of the Lymphocystis Disease of Fish is a True Virus. Anat. Rec., 111: (Abstract no. 291).
First filtration experiment supporting viral theory for lymphocystis. One *Fundulus* out of 31 specimens became infected when treated with *Stizostedion* tumor material filtered through Chamberland-Pasteur filter L5; one *Fundulus* out of 41 fish treated with non-filtered pike-perch material also became infected.

81. 1952. Parallel Features in the Parasitism and Life Cycle of Fish Microsporidia and Fish Viruses. Proc. Soc. Protozoologists, 3: (Abstract no. 5).
A new analysis of hypertrophy of tissue cells induced by microsporidians and by lymphocystis virus.

82. 1955. The Third Spontaneous Case of Lymphocystis Virus Disease of Fish from the Pacific Coast of North America. Anat. Rec., 122: 434-435 (Abstract no. 38).
Disease in *Hypsoblennius*, probably *H. jenkinsi*, from San Diego Bay, California.

83. 1956. Granular Components of the Basophilic Lattice in the Lymphocystis Virus Inclusion Bodies of *Pleuronectes*. Archiv f. die Gesamte Virusforschung, 7: 1-17.
Studies on flounders *Pleuronectes flesus* with spontaneously developed lesions from the Baltic in the vicinity of Rugen Island and in experimentally infected fish kept in aquaria. A basophilic lattice functions as a supporting framework for the maturing lymphocystis inclusion bodies. The relationships of the basophilic granules to the lattice and the fate of the lattice in advanced stages of the lymphocystis cell are discussed in relation to the filamentous virus resembling the influenza group.

84. 1960a. Some Remarkable Osmiophilic Structures of the Inclusion Bodies in the Lymphocystis Virus Disease of the European Flounder. Arch. f. die Gesamte Virusforschung, 10: 253-263.
Diseased flounders from Morecambe Bay, Irish Sea. Three types of osmiophilic structures are demonstrated: granules, paired dumb-bell

shaped rods (lying parallel or crossed) and lattice. These represent developmental stages of the lymphocystis virus. The elongated forms are considered to be the infectious stage, since they are found in great numbers in lymphocystis cell in an advanced stage of development.

85. 1960b. Paired Structures of the Inclusion Bodies in the Lymphocystis Disease of Perches. Bact. Proc. (Abstract no. M180).

Osmiophilic granules in the inclusion bodies of the pike-perch appear as tetrads in relatively young cells (250 microns). The tetrads consist of two symmetrical dyads connected by a delicate thread and sometimes by a denser filament, appearing as two rods with polar granules. Somewhat similar granules are found in lymphocystis cells of *Acerina cernua*. It is suggested that the virus of lymphocystis is filamentous.

86. 1960c. Further Studies on the Lymphocystis Disease of Fish. Anat. Rec., 137: 400. (Abstract).

The filamentous-like rods with osmiophilic granules at the tips are described and their distribution in the lymphocystis cells in several fish species indicated. In the tumors of the European flounder and perches, the rods are predominantly found within the cytoplasmic inclusion bodies; in the Atlantic angelfishes, they are found within the nucleus and as free intracytoplasmic colonies; paired rods were also found in leucocytes, apparently the result of phagocytosis.

87. 1965. Fifty Years of Research on the Lymphocystis Virus Disease of Fishes (1914-1964). *In:* Viral Diseases of Poikilothermic Vertebrates. Annals N.Y. Academy of Science, 126: 362-374.

Review of Dr. Weissenberg's research on lymphocystis disease from 1914, when he first proposed the virus theory, to the present day.

88. 1965. Morphological Studies on the Lymphocystis Tumor Cells of a Cichlid from Guatemala, *Cichlasoma synspilum* Hubbs. *ibid.*, 126: 396-413.

The young lymphocystis cell contains scattered basophilic corpuscles in contrast to single Guarnieri-like body that produces the inclusions seen in perches and flounder tumor cells. Vacuoles appear in the cytoplasm of young cell on the walls of which small groups of osmiophilic granules, filaments and sometimes paired rods accumulate. Electronmicroscopic studies show typical lymphocystis viral structures (polyhedral) in the cytoplasm.

89. Weissenberg, R., R. F. Nigrelli & G. M. Smith

1937. Lymphocystis in the Hogfish, *Lachnolaimus maximus*. Zoologica, 22: 303-305.

Description of the lymphocystis cell, together with an excellent drawing of a mature cell.

90. Wenyon, C. M.

1926. Protozoology. Vol. 1. William Wood & Co., New York. 788 pp.

Pages 770-773 refer to *Lymphocystis macropodis*, a sarcocystis-like parasite in the intestinal mucosa of the kangaroo *(Macropus* sp.); not to be confused with lymphocystis disease in the paradisefish, *Macropodus*.

91. Witt, A., Jr.

1957. Seasonal Variation in the Incidence of Lymphocystis in the White Crappie from the Niangua Arm of the Lake of the Ozarks, Missouri. Trans. Amer. Fish. Soc., 85: 271-279.

Total of 7,499 fish collected in 1950-1951 and in 1952-1955. Seasonal variation in incidence of the disease is as follows: July 10.7%, October 1.7%, November 6.9%, April 1.4%. The lesions are found on tail fins in most (60%) of the diseased fish; 97% of the infected fish collected in 1950-1951 were under 6.5 inches long (3 yr.-old, or 1949 class). The disease is not lethal but affected fish weighed 3-5% less than healthy specimens of the same length. One walleye in the collection was also infected, but no other centrarchid.

92. Wolf, Ken

1958. Lymphocystis Disease of Fish. U. S. Dept. Interior, Fish and Wildlife Service, Fishery Leaflet No. 458, 4 pages.

A brief description of the disease for fishery biologists, together with a selected annotated bibliography.

93. 1962. Experimental Propagation of Lymphocystis Disease of Fishes. Virology, 18: 249-256.

Experimental transmission of the lymphocystis virus from large-mouth bass to bluegill sunfish and propagated in the latter species for two years by implantation and by injection of filtered (Millipore HA) fresh, aged (2 yrs. at −20°C) or desiccated material. The virus is glycerol- and ether-sensitive.

94. Wolf, Ken, & C. P. Carlson

1965. Multiplication of Lymphocystis Virus in the Bluegill Sunfish *(Lepomis macrochirus)*. *In:* Viral Diseases of Poikilothermic Vertebrates. Annals N. Y. Acad. Sci., 126: 414-419.

Classical curve of multiplication at 25° C was demonstrated for the lymphocystis virus in experimental infections in bluegill sunfish.

95. Woodcock, H. M.

1904. Notes on a Remarkable Parasite of Plaice and Flounders. Trans. Liverpool Biol. Soc., 18: 143-152.

First description of the lymphocystis cell, believed to be a sporozoan parasite, for which the name *Lymphocystis johnstonei* was proposed.

96. Zschiesche, A.
1910. Eizellen in der Haut von Macropoden. Zool. Ans., 36: 294-298.

First to report the disease in the freshwater paradisefish, *Macropodus,* originally from China. However, the enlarged cells were thought to be eggs.

EXPLANATION OF THE PLATES

Plate I

1. Typical lymphocystis nodules in the dorsal fin of the disc cichlid, *Symphysodon discus.* 4×.
2. Nodules on dorsal fin of West Indian cowfish, *Lactophrys tricornis.* Note individual lymphocystis cells within the nodule and along several of the fin rays. 4×.
3. Lymphocystis nodules in the anal fin of the scat, *Scatophagus argus.* 2×.
4. Lymphocystis disease in the sleeper, *Dormitator maculatus.* Slightly less than natural size.

Plate II

5. Lymphocystis lesions in the European perch or ruff, *Acerina cernua,* one of the species in which the disease was first described by Dr. Weissenberg in 1914. Slightly larger than natural size. Courtesy of Dr. R. Weissenberg.
6. Lymphocystis "tumors" in the pike-perch, *Stizostedion vitreum,* the species in which the disease was first reported in North America by Mavor & Feinberg in 1918. About natural size.

Plate III

7. Typical fin lesions in the cichlid *Aequidens portalegrensis.* This fish was the donor for the transmission experiments reported in Table II and shown in Fig. 8.
8. *Aequidens portalegrensis* showing typical skin response to experimental infection. Natural size.

Plate IV

9. *Forcipiger longirostris* (forceps fish), showing characteristic tumor-like growth at the site in which two crushed lymphocystis cells from the right pectoral fin were introduced. Note the cells on the left pectoral fin. About natural size.
10. Lymphocystis disease in white perch, *Morone americana.* The hemorrhagic appearance is a characteristic response in the early stages of the disease. Note absence of nodules. About ½ natural size.
11. A non-nodular response of lymphocystis disease in the striped bass, *Roccus lineatus.* The individual hypertrophied connective tissue cells are scattered just below the epidermis. 2×.

Plate V

12. An exceptional development of lymphocystis cells on the dorsal surface of striped bass. The granular lesions appear as extensive thickened, yellowish patches. 3×.
13. Lymphocystis cells from the nodule in the cowfish shown in Fig. 2. Note the appearance of the nucleus and the cytoplasmic inclusions and compare with Figs. 15-24. Masson's stain; 150×.
14. A typical lymphocystis cell in the gill of the cowfish, showing the enlarged nucleolus, peripherally-scattered cytoplasmic inclusions and thickened hyalin membrane. The tissue reaction suggests that this cell developed *in situ,* and is not a metastatic element. Masson's stain; 350×.

Plate VI

15. Lymphocystis cells from forceps fish shown in Fig. 9. Haematoxylin-eosin; 150×.
16. Another area of the growth in Fig. 15 showing a binucleate cell, chromatin clumps within the nucleus and basophilic cytoplasmic inclusions. Note the gelatinous matrix. Haematoxylin-eosin; 300×.

Plate VII

17. Two characteristic hypertrophied cells from the lymphocystis disease in the forceps fish. The bottom cell measures 75 × 95 microns; nucleus 36 × 35 microns; nucleolus about 12 microns. Haematoxylin-eosin; 600×.
18. Cell from forceps fish as seen in the interference microscope. Note the plaques filled with weakly-staining bodies. Haematoxylin-eosin; 800×.

Plate VIII

19. Nuclear details of a single hypertrophied cell in the forceps fish. Note the dissolution of the nucleolus. Haematoxylin-eosin; 1350×.
20. Lymphocystis cells from white perch shown in Fig. 10. There is some shrinkage from fixation. In this species, as will be noted, the basophilic inclusions form compact masses in the periphery of the cell; the nucleus is highly vacuolated. Masson's stain; 300×.
21. Nuclear and cytoplasmic details in a single white perch lymphocystis cell. Haematoxylin-eosin; 1350×.

Plate IX

22. Inflammatory response associated with the lymphocystis disease in the white perch. Masson's stain; 300×.
23. A group of hypertrophied cells in experimentally induced lymphocystis disease in *Aequidens portalegrensis* shown in Fig. 8. The irregular shape of the cells is not due to shrinkage. Note that the capsular membrane is intact and follows the shape of the cell. Haematoxylin-eosin; 600×.
24. Details in the nuclear area of a lymphocystis cell from *Aequidens.* Note numerous granules each surrounded by a halo-like structure. Haematoxylin-eosin; interference microscope; 2000×.

Plate X

25. Lymphocystis cells in striped bass showing the extensive and unusual development of hyalin substance. Haematoxylin-eosin; 50×.
26. PAS-positive hyalin membrane surrounding the individual lymphocystis cell in white perch. This membrane or capsule is best seen in Fig. 14. 150×.